

WHAT IS CLAIMED IS:

1. An isolated nucleic acid molecule comprising a nucleotide
sequence selected from the group of nucleotide sequences consisting of an attB1
nucleotide sequence as set forth in Figure 9, an attB2 nucleotide sequence as set
forth in Figure 9, an attP1 nucleotide sequence as set forth in Figure 9, an attP2
nucleotide sequence as set forth in Figure 9, an attL1 nucleotide sequence as set
forth in Figure 9, an attL2 nucleotide sequence as set forth in Figure 9, an attR1
nucleotide sequence as set forth in Figure 9, an attR2 nucleotide sequence as set
forth in Figure 9, a polynucleotide complementary thereto, and a mutant,
fragment, or derivative thereof.

2. An isolated nucleic acid molecule comprising an attB1 nucleotide
sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a
mutant, fragment, variant or derivative thereof.

3. An isolated nucleic acid molecule comprising an attB2 nucleotide
sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a
mutant, fragment, variant or derivative thereof.

4. An isolated nucleic acid molecule comprising an attP1 nucleotide
sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a
mutant, fragment, variant or derivative thereof.

5. An isolated nucleic acid molecule comprising an attP2 nucleotide
sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a
mutant, fragment, variant or derivative thereof.

6. An isolated nucleic acid molecule comprising an attL1 nucleotide
sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a
mutant, fragment, variant or derivative thereof.

7. An isolated nucleic acid molecule comprising an attL2 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

5 8. An isolated nucleic acid molecule comprising an attR1 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

10 9. An isolated nucleic acid molecule comprising an attR2 nucleotide sequence as set forth in Figure 9, a polynucleotide complementary thereto, or a mutant, fragment, variant or derivative thereof.

15 10. The isolated nucleic acid molecule of claim 1, further comprising one or more functional or structural nucleotide sequences selected from the group consisting of one or more multiple cloning sites, one or more localization signals, one or more transcription termination sites, one or more transcriptional regulatory sequences, one or more translational signals, one or more origins of replication, one or more fusion partner peptide-encoding nucleic acid molecules, one or more protease cleavage sites, and one or more 5' polynucleotide extensions.

20 11. The nucleic acid molecule of claim 10, wherein said transcriptional regulatory sequence is a promoter, an enhancer, or a repressor.

25 12. The nucleic acid molecule of claim 10, wherein said fusion partner peptide-encoding nucleic acid molecule encodes glutathione S-transferase (GST), hexahistidine (His₆), or thioredoxin (Trx).

30 13. The nucleic acid molecule of claim 10, wherein said 5' polynucleotide extension consists of from one to five nucleotide bases.

14. The nucleic acid molecule of claim 13, wherein said 5' polynucleotide extension consists of four or five guanine nucleotide bases.

5 15. A primer nucleic acid molecule suitable for amplifying a target nucleotide sequence, comprising the isolated nucleic acid molecule of claim 1 or a portion thereof linked to a target-specific nucleotide sequence useful in amplifying said target nucleotide sequence.

10 16. The primer nucleic acid molecule of claim 15, wherein said primer comprises an attB1 nucleotide sequence having the sequence shown in Figure 9 or a portion thereof, or a polynucleotide complementary to the sequence shown in Figure 9 or a portion thereof.

15 17. The primer nucleic acid molecule of claim 15, wherein said primer comprises an attB2 nucleotide sequence having the sequence shown in Figure 9 or a portion thereof, or a polynucleotide complementary to the sequence shown in Figure 9 or a portion thereof.

20 18. The primer nucleic acid molecule of claim 15, further comprising a 5' terminal extension of four or five guanine bases.

19. A vector comprising the isolated nucleic acid molecule of claim 1.

25 20. The vector of claim 19, wherein said vector is an Expression Vector.

30 21. A host cell comprising the isolated nucleic acid molecule of claim 1 or the vector of claim 19.

22. A method of synthesizing or amplifying one or more nucleic acid molecules comprising:

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- (a) mixing one or more nucleic acid templates with at least one polypeptide having polymerase or reverse transcriptase activity and at least a first primer comprising a template-specific sequence that is complementary to or capable of hybridizing to said templates and at least a second primer comprising all or a portion of a recombination site wherein said at least a portion of said second primer is homologous to or complementary to at least a portion of said first primer; and
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- (b) incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates and comprising one or more recombination sites or portions thereof at one or both termini of said molecules.
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23. A method of synthesizing or amplifying one or more nucleic acid molecules comprising:

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- (a) mixing one or more nucleic acid templates with at least one polypeptide having polymerase or reverse transcriptase activity and at least a first primer comprising a template-specific sequence that is complementary to or capable of hybridizing to said templates and at least a portion of a recombination site, and at least a second primer comprising all or a portion of a recombination site wherein said at least a portion of said recombination site on said second primer is complementary to or homologous to at least a portion of said recombination site on said first primer; and
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- (b) incubating said mixture under conditions sufficient to synthesize or amplify one or more nucleic acid molecules complementary to all or a portion of said templates and comprising one or more
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recombination sites or portions thereof at one or both termini of said molecules.

5 24. A method of amplifying or synthesizing one or more nucleic acid molecules comprising:

- 10 (a) mixing one or more nucleic acid templates with at least one polypeptide having polymerase or reverse transcriptase activity and one or more first primers comprising at least a portion of a recombination site and a template-specific sequence that is complementary to or capable of hybridizing to said template;
- 15 (b) incubating said mixture under conditions sufficient to synthesize or amplify one or more first nucleic acid molecules complementary to all or a portion of said templates wherein said molecules comprise at least a portion of a recombination site at one or both termini of said molecules;
- 20 (c) mixing said molecules with one or more second primers comprising one or more recombination sites, wherein said recombination sites of said second primers are homologous to or complementary to at least a portion of said recombination sites on said first nucleic acid molecules; and
- 25 (d) incubating said mixture under conditions sufficient to synthesize or amplify one or more second nucleic acid molecules complementary to all or a portion of said first nucleic acid molecules and which comprise one or more recombination sites at one or both termini of said molecules.

 25. A polypeptide encoded by the isolated nucleic acid molecule of any one of claims 1-10.

30 26. An isolated nucleic acid molecule comprising one or more *att* recombination sites comprising at least one mutation in its core region that

increases the specificity of interaction between said recombination site and a second *att* recombination site.

5 27. The isolated nucleic acid molecule of claim 26, wherein said mutation is at least one substitution mutation of at least one nucleotide in the seven basepair overlap region of said core region of said recombination site.

10 28. The isolated nucleic acid molecule of claim 26, wherein said nucleic acid molecule comprises the sequence NNNATAC, wherein "N" refers to any nucleotide with the proviso that if one of the first three nucleotides in the consensus sequence is a T/U, then at least one of the other two of the first three nucleotides is not a T/U.

15 29. An isolated nucleic acid molecule comprising one or more mutated *att* recombination sites comprising at least one mutation in its core region that enhances the efficiency of recombination between a first nucleic acid molecule comprising said mutated *att* recombination site and a second nucleic acid molecule comprising a second recombination site that interacts with said mutated *att* recombination site.

20 30. The isolated nucleic acid molecule of claim 29, wherein said mutated *att* recombination site is a mutated *attL* site comprising a core region having the nucleotide sequence caactntntnnannaagttg (SEQ ID NO:92), wherein "n" represents any nucleotide.

25 31. The isolated nucleic acid molecule of claim 30, wherein said mutated *attL* recombination site comprises a core region having a nucleotide sequence selected from agcctgctttattataactaagttggcatta (*attL5*; SEQ ID NO:87) and agcctgctttttatattaagttggcatta (*attL6*; SEQ ID NO:88).

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32. The isolated nucleic acid molecule of claim 29, wherein said mutated *att* recombination site comprises a core region having a nucleotide sequence selected from the group consisting of ggggacaactttgtacaaaaaagttggct (*att*B1.6; SEQ ID NO:105), ggggacaactttgtacaagaaagctgggt (*att*B2.2; SEQ ID NO:97), and ggggacaactttgtacaagaaagttgggt (*att*B2.10; SEQ ID NO:107).

33. A vector selected from the group consisting of pENTR1A, pENTR2B, pENTR3C, pENTR4, pENTR5, pENTR6, pENTR7, pENTR8, pENTR9, pENTR10, pENTR11, pDEST1, pDEST2, pDEST3, pDEST4, pDEST5, pDEST6, pDEST7, pDEST8, pDEST9, pDEST10, pDEST11, pDEST12.2 (also known as pDEST12), pDEST13, pDEST14, pDEST15, pDEST16, pDEST17, pDEST18, pDEST19, pDEST20, pDEST21, pDEST22, pDEST23, pDEST24, pDEST25, pDEST26, pDEST27, pDEST28, pDEST29, pDEST30, pDEST31, pDEST32, pDEST33, pDEST34, pDONR201 (also known as pENTR21 attP vector or pAttPkan Donor Vector), pDONR202, pDONR203 (also known as pEZ15812), pDONR204, pDONR205, pDONR206 (also known as pENTR22 attP vector or pAttPgen Donor Vector), pDONR207, pMAB58, pMAB62, pMAB85 and pMAB86.

34. A host cell comprising the vector of claim 33.

35. A polypeptide encoded by the vector of claim 33.

36. A kit for use in synthesizing a nucleic acid molecule, said kit comprising the isolated nucleic acid molecule of any one of claims 1-10, 26 and 29.

37. A kit for use in synthesizing a nucleic acid molecule, said kit comprising the primer of claim 15 or claim 18.

38. A kit for use in cloning a nucleic acid molecule, said kit comprising the vector of claim 19 or claim 33.